REMARKS

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date 11/22/02

By Paul J. White

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MARKED UP VERSION ATTACHED TO AMENDMENT IN

SERIAL NO. 09/917,384

Marked up version of the paragraph starting at page 9, lines 18-30, is below:

"Fusion protein" refers to a first protein having attached a second, heterologous protein. Preferably, the heterologous protein is fused via recombinant DNA techniques, such that the first and second proteins are expressed in frame. The heterologous protein can confer a desired characteristic to the fusion protein, for example, a detection signal, enhanced stability or stabilization of the protein, facilitated oligomerization of the protein, or facilitated purification of the fusion protein. Examples of heterologous proteins useful in the fusion proteins of the invention include molecules having one or more catalytic domains of Gux1, one or more binding domains of Gux1, one or more catalytic domains of a glycoside hydrolase other than Gux1, one or more binding domains of a glycoside hydrolase other than Gux1, or any combination thereof. Further examples include immunoglobulin molecules and portions thereof, peptide tags such as histidine tag (6-His) **(SEQ ID NO: 8)**, leucine zipper, substrate targeting moieties, signal peptides, and the like. Fusion proteins are also meant to encompass variants and derivatives of Gux1 polypeptides that are generated by conventional site-directed mutagenesis and more modern techniques such as directed evolution, discussed infra.

Marked up version of the paragraph starting at page 20, lines 4-10, is below:

Gux1 polypeptides can be fused to heterologous polypeptides to facilitate purification. Many available heterologous peptides (peptide tags) allow selective binding of the fusion protein to a binding partner. Non-limiting examples of peptide tags include 6-His **(SEQ ID NO: 8)**, thioredoxin, hemaglutinin, GST, and the OmpA signal sequence tag. A binding partner that recognizes and binds to the heterologous peptide can be any molecule or compound, including metal ions (for example, metal affinity columns), antibodies, antibody fragments, or any protein or peptide that preferentially binds the heterologous peptide to permit purification of the fusion protein.

Marked up version of the table starting at page 33, is below:

Table 3. Multiple amino acid sequence alignment of a Gux1 first catalytic domain and polypeptides with Glycoside Hydrolase Family 48 catalytic domains.

Multialignment of related Glycoside Hydrolase Family 48 catalytic domain
GH48_Ace: *Acidothermus cellulolyticus* Gux1 catalytic domain GH48 (SEQ ID NO: 9)
GuxB_Cfi: Cellulomonas fimi CBHB(beta-1,4-exocellobiohydrolase). GeneBank Acc. # AAB00822 (SEQ ID NO: 10)
E6_Tfu: Thermobifida fusca E6 (beta-1,4-exocellulase). GeneBank Acc. # AF144563 (SEQ ID NO: 11)

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GH48_Ace        PYIQRFLTMYNKIHDPANGYFSPQG---IPYHSVETLIVEAPDYGHETTSEAYSFWLWLEATY (SEQ ID NO:9)
GuxB_Cfi        EYAQRFLAQYDKIKDPANGYFSAQG---IPYHAVETLMVEAPDYGHETTSEAYSYWLWLEALY (SEQ ID NO:10)
E6_Tfu          SYDQAFLEQYEKIKDPASGYFREFNGLLVPYHSVETMIVEAPDHGHQTTSEAFSYYLWLEAYY (SEQ ID NO:11)
                 * * **  *:**:***.***    .   :***:***::*****:**:*****:*::***** *

GH48_Ace        GAVTGNWTPFNNAWTTMETYMIPQHADQPNNASYNPNSPASYAPEEPLPSMYPVAIDSSV
GuxB_Cfi        GQVTQDWAPLNHAWDTMEKYMIPQSVDQPTNSFYNPNSPATYAPEFNHPSSYPSQLNSGI
E6_Tfu          GRVTGDWKPLHDAWESMETFIIPGTKDQPTNSAYNPNSPATYIPEQPNADGYPSPLMNNV
                * ** :* *::.** :**.::**   ***.*: *******:* **   .. **  : ..:

GH48_Ace        PVGHDPLAAELQSTYGTPDIYGMHWLADVDNIYGYGDSPGGGCELGPSAKGVSYINTFQR
GuxB_Cfi        SGGTDPIGAELKATYGNADVYQMHWLADVDNIYGFGATPGAGCTLGPTATGTSFINTFQR
E6_Tfu          PVGQDPLAQELSSTYGTNEIYGMHWLLDVDNVYGFGFCGDG------TDDAPAYINTYQR
                . * **:. **.:***. ::* **** ****:**:*   ..      :  . ::***:**

GH48_Ace        GSQESVWETVTQPTCDNGKYGGAHGYVDLFIQG-STPPQWKYTDAPDADARAVQAAYWAY
GuxB_Cfi        GPQESVWETVPQPSCEEFKYGGKNGYLDLFTKDASYAKQWKYTSASDADARAVEAVYWAN
E6_Tfu          GARESVWETIPHPSCDDFTHGGPNGYLDLFTDDQNYAKQWRYTNAPDADARAVQVMFWAH
                *.:******:.:*:*:: .:** :**:*** .. . . **:**.*.*******:. :**

GH48_Ace        TWASAQGKASAIAPTIAKASQTGDYLRYSLFDKYFKQVGNCYPASSCPGATGRQSETYLI
GuxB_Cfi        QWATEQGKAADVAATVAKAAKMGDYLRYTLFDKYFKKIG--CTSPTCAAGQGREAAHYLL
E6_Tfu          EWAKEQGKENEIAGLMDKASKMGDYLRYAMFDKYFKKIGNCVGATSCPGGQGKDSAHYLL
                 **. ***   :*  : **:: ******::******::*    :.:*... *::: **:

GH48_Ace        GWYYAWGGS---SQGWAWRIGDGAAHFGYQNPLAAWAMSNVTPLIPLSPTAKSDWAASLQ
GuxB_Cfi        SWYMAWGGATDTSSGWAWRIGSSHAHFGYQNPLAAWALSTDPKLTPKSPTAKADWAASMQ
E6_Tfu          SWYYSWGGSLDTSSAWAWRIGSSSSHQGYQNVLAAYALSQVPELQPDSPTGVQDWATSFD
                .** :***:   *..******.. :* **** ***:*:*  . * * ***.  ***:*::

GH48_Ace        RQLEFYQWLQSAEGAIAGGATNSWNGNYGTPPAGDSTFYGMAYDWEPVYHDPPSNNWFGF
GuxB_Cfi        RQLEFYTWLQASNGGIAGGATNSWDGAYAQPPAGTPTFYGMGYTEAPVYVDPPSNRWFGM
E6_Tfu          RQLEFLQWLQSAEGGIAGGATNSWKGSYDTPPTGLSQFYGMYYDWQPVWNDPPSNNWFGF
                ***** ***:::*.*********.* *  **:* . **** *   **: *****.***:

GH48_Ace        QAWSMERVAEYYYVTGDPKAKALLDKWVAWVKPNVTTG-----ASWSIPSNLSWSGQPDT
GuxB_Cfi        QAWGVQRVAELYYASGNAQAKKILDKWVPWVVANISTDG----ASWKVPSELKWTGKPDT
E6_Tfu          QVWNMERVAQLYYVTGDARAEAILDKWVPWAIQHTDVDADNGGQNFQVPSDLEWSGQPDT
                *.*.::***: **.:*:.:*: :*****.*.  :  ..      .:.:**:*.*:*:***

GH48_Ace        WNPSNPGTNANLHVTITSSGQDVGVAAALAKTLEYYAAKSGDTASRDLAKGLLDSMWNND
GuxB_Cfi        WNAAAPTGNPGLTVEVTSYGQDVGVAADTARALLFYAAKSGDTASRDKAKALLDAIWANN
E6_Tfu          WTG-TYTGNPNLHVQVVSYSQDVGVTAALAKTLMYYAKRSGDTTALATAEGLLDALLAHR
                *.      *..* * :.* .*****:*  *::* :** :****::   *:.***::  :

GH48_Ace        QDSLGVSTPETRTDYSRFTQVYDPTTGDGLYIPSGWTGTMPNGDQIKPGATFLSIRSWYT
GuxB_Cfi        QDPLGVSAVETRGDYKRFDDTYVAN-GDGIYIPSGWTGTMPNGDVIKPGVSFLDIRSFYK
E6_Tfu          -DSIGIATPEQ-PSWDRLDDPWDGS--EGLYVPPGWSGTMPNGDRIEPGATFLSIRSFYK
                 *.:*::: *   .:.*: : :  .  :*:*:*.**:******* *:**.:**.***:*.

GH48_Ace        KDPQWSKVQAYLNGG---PAPTFNYHRFWAESDFAMANADFGMLFPSGSP
GuxB_Cfi        KDPNWSKVQTFLDGG---AEPQFRYHRFWAQTAVAGALADYARLFDDGTT
E6_Tfu          NDPLWPQVEAHLNDPQNVPAPIVERHRFWAQVEIATAFAAHDELFGAGAP
                :** *.:*::.*:.    . * .. *****:  .* * * .  **  *:.
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In the Claims:

Please amend the claims as follows:

24. (Amended) The composition of claim 23 wherein the peptide tag is 6-His **(SEQ ID NO: 8)**, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

32. (Amended) The fusion protein of claim 31, wherein the peptide tag is 6-His **(SEQ ID NO: 8)**, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

Marked-up set of claims for 09/917,384

We claim:

1. A composition comprising a substantially purified thermostable Gux1 peptide, said Gux1 peptide comprising a catalytic domain GH48, a carbohydrate binding domain (CBD) type III, and a carbohydrate binding domain (CBD) type II.

2. The composition of claim 1 wherein the Gux1 peptide is further defined as comprising a linker and a signal peptide.

3. The composition of claim 1 or 2 wherein the GH48 catalytic domain of the Gux1 peptide is further defined as having a length of about 637 to about 643 amino acids.

4. The composition of claim 1, 2, or 3 wherein the carbohydrate binding domain (CBD) type III of the Gux1 peptide is further defined as having a length of about 150 to about 156 amino acids.

5. The composition of claim 1, 2, 3, or 4 wherein the carbohydrate binding domain (CBD) type II of the Gux1 peptide is further defined as having a length of about 95 amino acids to about 105 amino acids in length.

6. The composition of claim 3 wherein the GH48 catalytic domain is further defined as the sequence of SEQ ID NO: 5.

7. The composition of claim 4 wherein the carbohydrate binding domain (CBD) type III is further defined as the sequence of SEQ ID NO: 4.

8. The composition of claim 6 wherein the carbohydrate binding domain (CBD) type II is further defined as the sequence of SEQ ID NO:7.

9. The composition of claim 1 further defined as comprising a sequence of SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 7.

10. A thermal tolerant Gux1 peptide having a sequence of SEQ ID NO: 1.

11. The Gux1 peptide of claim 10 further defined as having a sequence of SEQ ID NO: 2.

12. (Cancelled) An industrial mixture suitable for degrading cellulose, such mixture comprising the Gux1 polypeptide of claim 1.

13. (Cancelled) The industrial mixture of claim 12 further defined as comprising a detergent.

14. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

15. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 80% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

16. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 70% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

17. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 7.

18. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 4.

19. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 6.

20. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1.

21. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of SEQ ID NO: 2.

22. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence encoding a heterologous protein in frame with the Gux1 peptide of claim 1.

23. The composition of claim 22 wherein the heterologous protein in frame with the Gux1 peptide of claim 1 is further defined as a peptide tag.

24. (Amended) The composition of claim 23 wherein the peptide tag is 6-His **(SEQ ID NO: 8)**, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

24. The composition of claim 22 wherein the heterologous protein is a substrate targeting moiety.

25. The composition of claim 13 wherein the nucleotide sequence encoding the Gux1 is operably linked to a transcriptional or translational regulatory sequence.

26. The composition of claim 25, wherein the transcriptional or translational regulatory sequence comprises a transcriptional promoter or enhancer.

27. An isolated polypeptide molecule comprising:

a) a sequence of SEQ ID NO: 4;

b) a sequence of SEQ ID NO: 5;

c) a sequence of SEQ ID NO: 6;

d) a sequence of SEQ ID NO: 7;

e) a sequence of SEQ ID NO: 1; or

f) an amino acid sequence having at least 70% sequence identity with the amino acid sequence of a), b), c), d), or e).

28. The polypeptide molecule of claim 27, having at least 90% sequence identity with the amino acid sequence of a), b), c), d), or e).

29. A fusion protein comprising the polypeptide of claim 27 and a heterologous peptide.

30. The fusion protein of claim 29, wherein the heterologous peptide is a substrate targeting moiety.

31. The fusion protein of claim 29, wherein the heterologous peptide is a peptide tag.

32. The fusion protein of claim 31, wherein the peptide tag is 6-His **(SEQ ID NO: 8)**, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

33. The fusion protein of claim 29, wherein the heterologous peptide is an agent that promotes polypeptide oligomerization.

34. The fusion protein of claim 29, wherein the agent is a leucine zipper.

35. (Cancelled) A cellulase-substrate complex comprising the isolated polypeptide molecule of claim 27 bound to cellulose.

36. (Cancelled) A vector comprising the polynucleotide molecule that encodes a polypeptide of claim 27.

37. (Cancelled) A host cell genetically engineered to express the polypeptide molecule of claim 27.

38. (Cancelled) A host cell genetically engineered to express the polynucleotide molecule of claim 27.

39. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a plant cell.

40. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a fungi.

41. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.

42. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.

43. A composition comprising the polypeptide molecule of claim 27 and a carrier.

44. A composition comprising the polypeptide molecule of claim 28 and a carrier.

45. (Cancelled) An isolated antibody that specifically binds to the polypeptide molecule of claim 27.

46. (Cancelled) The antibody of claim 45, wherein the antibody is a polyclonal antibody.

47. (Cancelled) The antibody of claim 45, wherein the antibody is a monoclonal antibody.

48. (Cancelled) A method for producing Gux1 polypeptide, the method comprising:

incubating a host cell genetically engineered to express the polynucleotide molecule of claim 27.

49. (Cancelled) The method of claim 48, further comprising the step of:

isolating the Gux1 polypeptide from the incubated host cells.

50. (Cancelled) The method of claim 48, wherein the host cell is a plant cell.

51. (Cancelled) The method of claim 48, wherein the host cell is a bacterial cell.

52. (Cancelled) The method of claim 48, wherein the host cell is genetically engineered to express a selectable marker.

53. (Cancelled) The method of claim 48, wherein the host cell further comprises a polynucleotide molecule encoding one or more polypeptide molecules selected from the glycoside hydrolase family of proteins.

54. (Cancelled) The method of claim 53, wherein the glycoside hydrolase is a thermostable glycoside hydrolase.

55. (Cancelled) A set of amplification primers for amplification of a polynucleotide molecule encoding Gux1, comprising:

two or more sequences comprising 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.

56. (Cancelled) A probe for hybridizing to a polynucleotide encoding Gux1, comprising:

a sequence of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.

57. (Cancelled) An assay method for the detection of a polynucleotide encoding Gux1, comprising:

amplifying a nucleic acid sequence with a set of amplification primers comprising two or more sequences of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27; and

correlating the amplified nucleic acid sequence with detected polypeptide encoding Gux1.

58. (Cancelled) A method for assessing the carbohydrate degradation activity of Gux1 comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the absence of Gux1 on a substrate; and

comparing the carbohydrate degradation in the presence of Gux1 with the carbohydrate degradation in the absence of Gux1.

59. (Cancelled) A method for assessing the carbohydrate degradation activity of Gux1 in the presence of an agent of interest comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the presence of Gux1 and the agent of interest on a substrate exposed; and

comparing the carbohydrate degradation in the Gux1 treated substrate with the carbohydrate degradation in the Gux1 treated substrate in the presence of the agent of interest.

60. (Cancelled) The method of claim 59, wherein an increase in carbohydrate degradation activity in the presence of the agent of interest demonstrates stimulation of Gux1 activity and wherein a decrease in carbohydrate degradation activity demonstrates inhibition of Gux1 activity.

61. (Cancelled) The method of claim 58, wherein the carbohydrate is cellulose.

62. (Cancelled) The method of claim 58 wherein the agent of interest is an antibody.

63. (Cancelled) A method for reducing cellulose in a starting material, the method comprising:

administering to the starting material an effective amount of a polypeptide molecule of claim 27.

64. (Cancelled) The method of claim 63, further comprising administering a second polypeptide molecule selected from the glycoside hydrolase family of proteins.

65. (Cancelled) The method of claim 63, wherein the polypeptide molecule of claim 27 is thermostable.

66. (Cancelled) The method of claim 63, wherein the starting material is agricultural biomass.

67. (Cancelled) The method of claim 63, wherein the starting material is municipal solid waste.

68. (Added) The composition of claim 1 further comprising a carrier.

69. (Added) The composition of claim 1 wherein the substantially purified thermostable Gux I peptide is further defined as comprising a heterologous peptide or protein.

70. (Added) The composition of claim 69 wherein the heterologous peptide or protein comprises an immunoglobulin.

71. (Added) The composition of claim 69 wherein the heterologuos peptide comprises a histidine tag.

72. (Added) The composition of claim 69 wherein the heterologus peptide comprises a leucine zipper.

73. (Added) The composition of claim 69 wherein the heterologous peptide comprises a fusion protein.